Z. Säugetierkunde **63** (1998) 179–182

ZEITSCHRIFT FÜR SÄUGETIERKUNDE
INTERNATIONAL JOURNAL OF MAMMALIAN BIOLOGY

First results on genetic variability in an autochthonous population of Roe deer from a Mediterranean forest in southern Spain

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Receipt of Ms. 15. 08. 1997
Acceptance of Ms. 17. 11. 1997

Key words: *Capreolus*, genetic variability, southern Spain

The Cádiz and Málaga mountains in southern Spain constitute the southwestern limit of the distribution of the roe deer (*Capreolus capreolus* Linnaeus, 1758) (Aragón et al. 1995 a). In this region the species inhabits a Mediterranean xerophytic forest reaching densities from 1.6 to 10.3 individuals per 100 ha (Braza et al. 1994). Anatomical peculiarities have been recorded in this population, both in terms of external morphology (Aragón et al. 1995 b) and craniometry (Aragón et al. 1998), supporting the existence of a Mediterranean ecotype of roe deer distinguished by a dark gray winter fur which turns to reddish or grayish in summer, small size, and short and wide skulls (Braza et al. 1994).

To date, genetic variability in roe deer has been studied mostly in eastern and central European populations (Baccus et al. 1983; Hartl et al. 1991, 1993; Lorenzini et al. 1993, 1996; Wehner et al. 1991). Our aim is to provide the first results on genetic variability for a population living in a Mediterranean forest. There was an absence of introduction of allochthonous individuals in the area or reductions in numbers in recent times. For genetic comparisons, samples coming from north-eastern France (Trois Fontaines, Marne) were used.

A total of 43 blood samples was collected, 25 (18 males and 7 females) from Cádiz and 18 (6 males and 12 females) from Trois Fontaines. After capture of an animal, a blood sample was taken from the jugular vein using a syringe. EDTA was used for preventing coagulation. Aliquots of serum and cells were stored at –20 °C until electrophoresis. Preparation of cell extracts, gel making (starch gel 12%), electrophoretic conditions, and staining procedures followed standard protocols (Pasteur et al. 1987). The following loci were analysed (E.C. numbers are given in parentheses): Ldh-1, -2 (1.1.1.27), Mdh-1 (1.1.1.37), 6Pgd (1.1.1.44), Sod-1, -2 (1.15.1.1), Pk (2.7.1.40), Ak (2.7.4.3), heart-Est, serum-Est (3.1.1.1), Mpi (5.3.1.8), Gpi (5.3.1.9), Hb, and Alb. Results were interpreted following Harris and Hopkinson (1976). Genotype frequencies were obtained directly by scoring the gels.

Four out of 14 loci were polymorphic (Tab. 1). For these loci genotype frequencies were in agreement with the Hardy-Weinberg expectations only at Mdh-1 in Cádiz (Chi-square = 3.81, d. f. = 5, NS). For all other loci there was a deficiency in the number of heterozygotes ($p < 0.001$ for all loci). In Cádiz, no relation was found between the deviation from Hardy-Weinberg and sex or summer appearance of the animals (4 reddish and 12 grayish) (Fisher test for Hb and Pk, and G test for heart-Est). The percentage of poly-

Table 1. Allozyme variability at the polymorphic loci in two roe deer populations from Cádiz (southern Spain) and Trois Fontaines (north-eastern France). Allele frequencies (p), observed single locus heterozygosity (Ho), and expected heterozygosity (He).

Locus	Allele	Cádiz			Trois Fontaines		
		p	Ho	He	p	Ho	He
Mdh 1	A	0.812	0.208	0.312	1.0	0.0	0.0
	B	0.020			0.0		
	C	0.172			0.0		
Pk	A	0.350	0.0	0.455	0.700	0.0	0.420
	B	0.650			0.300		
Heart-Est	A	0.023	0.045	0.429	0.0	0.0	0.0
	B	0.704			1.0		
	C	0.273			0.0		
Hb	A	0.333	0.0	0.444	0.0	0.0	0.0
	B	0.667			1.0		

morphic loci (99% criterium) (P), the observed (Ho) and expected heterozygosity (He) and the mean number of alleles per locus (A) were (± standard errors): P = 28.57%, Ho = 0.018 ± 0.056, He = 0.117 ± 0.195, and A = 1.428 ± 0.755 for Cádiz, and: P = 7.14%, Ho = 0, He = 0.030 ± 0.112 and A = 1.071 ± 0.267 for Trois Fontaines.

Our results confirm that roe deer is one of the most polymorphic species of deer (Lorenzini et al. 1993, six species included). The mean value of polymorphism for Cádiz is one of the highest measured, ranging from 10.5% (Baccus et al. 1983) to 35.7% (Wehner et al. 1991). On the contrary, the degree of heterozygosity in Cádiz is one of the lowest described, ranging from 1.4% (Wehner et al. 1991) to 8.1% (Hartl et al. 1991). The low variability found in Trois Fontaines may be a consequence of human management of the population, living in a fenced area of 1,369 ha.

Habitat use and the social system of the species in areas with a low density of animals may explain the genetic pattern found in Cádiz. In such circumstances adult males exhibit marked territorial behaviour, excluding other males but overlapping with the foraging area of different females. Due to low densities, young males establish territories close to their relatives because free areas are availables (Bideau et al. 1985). This particular spatial pattern may favour inbreeding and, consequently, if the probability of mating with a relative is higher than expected at random, homozygote frequency will increase in the area (Ayala 1975). The genetic variability in the population is maintained by animals which do not find a place close to their relatives, bearing in mind that a single individual migrating among populations per generation is sufficient for preserving homogeneity in allele frequencies (Slatkin 1987).

Table 2. Nei's (1978) genetic identities and standard distances calculated for different populations of roe deer in Europe.

	Identities	Distances	References
Cádiz/T. Fontaines	0.9929	0.0071	Present study
Austria (5 populations)	0.9855–0.9981	0.019–0.0146	Hartl and Reimoser (1988)
Central Europe (20 populations)*	0.9774–1	0–0.0226	Hartl et al. (1991)
Italy (4 populations)	0.9870–0.9990	0.001–0.013	Lorenzini et al. (1993)

* Populations from Switzerland, Austria and Hungary.

Genetic distance and genetic identities, calculated following Nei (1978), are similar to those reported for other European populations (Tab. 2), so we conclude, in agreement with Lorenzini et al. (1993), that genetic patterns of differentiation in roe deer populations are caused by ecological and ethological traits, such as breeding biology and dispersal pattern, rather than being a consequence of the existence of genetically well-separated subspecies. However, the results are very preliminary and may be influenced by the low number of loci and individuals analysed.

Acknowledgements

We would like to thank the "Office National de la Chasse de France" for their invitation to the roe deer capturing season, specially Dr. J. M. Boutin and Dr. B. Boisaubert. Dr. R. Lorenzini for their comments to the manuscript. Laboratory work was done at "Museo Nacional de Ciencias Naturales" in Madrid (Spain), directed by Dr. L. Boto. S. Aragón was supported by the Spanish Minestry for Education and Science (Predoctoral grant).

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